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Influence of dietary protein on insulin-like growth factor binding proteins in the chicken☆

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Abstract

We determined the effect of dietary protein on the distribution of insulin-like growth factor (IGF) binding proteins in chicken plasma. Three groups of male broilers ($n = 6$ per group) were fed (ad libitum) isocaloric diets containing 12, 21 or 30% dietary protein. Birds were fed respective diets beginning at 7 days of age and killed at 28 days. No differences were observed between adequate (21%) and high (30%) protein intakes for any of the parameters investigated (growth criteria, plasma levels of IGF-I, growth hormone or IGF-binding proteins). Feeding protein deficient diets (12%) resulted in a 34% decrease in body weight, 17% decrease in feed intake and a 39% increase in feed/gain ratio. IGF-binding proteins in plasma samples were separated by SDS-PAGE and transferred to nitrocellulose sheets. Nitrocellulose blots were probed with [^{125}I]chicken IGF-II. Four regions of binding activity corresponding to 70, 43, 30 and 24 kDa were observed in all samples. Birds consuming 12% dietary group protein had less than 50% of the 43-kDa binding activity of birds consuming 21 or 30% dietary protein. The 30-kDa binding activity was 42% lower in the 12% dietary protein group compared to birds consuming adequate protein. In contrast, 70- and 24-kDa binding activities were not influenced by dietary protein. Chickens consuming 12% dietary protein had higher levels of growth hormone and lower levels of IGF-I than those consuming 21 or 30% dietary protein. These data indicate that in chickens, the circulating levels of at least two independent IGF-binding proteins are influenced by dietary protein.

Keywords: Insulin-like growth factors; Growth hormone; IGF-binding proteins; Chickens; Broilers; Dietary protein; Ligand blotting

1. Introduction

Insulin-like growth factors (IGFs) and growth hormone (GH) are polypeptide growth factors that are integrally involved in the regulation of growth, nutrient utilization and cellular differentiation. Compared to mammals, the circulating levels of GH are several-fold higher in chickens, while IGF concentrations are markedly lower [12,25].

The IGFs (IGF-I and IGF-II) in blood and other fluids are complexed with a family of at least six distinct high affinity IGF-binding proteins (IGF-BPs), which influence the availability and function of the IGFs [21]. Separation analysis of mammalian and chicken IGF-I binding proteins by molecular sieve liquid chromatography reveals two major peaks of IGF binding activity; one at approximately 150 kDa and a second peak at approximately 40 kDa [11]. When separated by electrophoresis, subjected to ligand blot analysis and probed with [^{125}I]IGF-I, at least three discrete bands of IGF binding activity are observed in chicken plasma, while in mammalian plasma, four binding proteins are readily identified [11]. Thus, mammalian and chicken serum IGF-I binding protein profiles are similar, but not identical. Additionally, the levels of IGF-binding proteins are considerably lower in chickens, probably due to lower overall levels of IGFs. In

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chicken serum, a unique IGF-II-specific binding protein(≈ 70 kDa) has been identified but not characterized [11,20]. Upon ligand blot analysis this IGF-II binding protein can only be visualized by probing with labeled IGF-II.

In mammals and poultry, circulating levels of IGF-I [16,17] and growth hormone [18] are regulated by protein intake. Animals fed low protein diets grow more slowly and have lower circulating levels of IGF-I, while growth hormone levels are elevated by consumption of low protein diets. When chickens were fed low protein diets, transient, but not long-term fluctuations in plasma IGF-binding activities were observed [9]. Additionally, in mammals, it has been shown that the IGF-binding protein designated as IGF-BP3 (corresponding to 43 kDa binding activity) is decreased, while some of the lower molecular weight binding species (IGF-BP1 and 2) are unaffected when rats are fed protein-free diets [22]. The objective of the present study was to determine the influence of low versus high protein nutriture on circulating levels IGF-I and IGF-binding proteins in rapid growing broiler chickens.

2. Materials and methods

2.1. Animals

At 7 days of age (140 g), male, Indian River broiler chicks were assigned to one of three dietary treatments ($n = 6$ per group) and received diets containing 120, 210 or 300 g protein/kg diet. Diets were isocaloric (12.8 MJ ME/kg) and contained equivalent amounts of sulfur-containing amino acids (10.3 g/kg). The composition and formulations for these soybean meal-corn-based diets have been previously described [17]. Chickens were housed (six per pen) in battery-brooders in an environmentally controlled room maintained at 23°C with a 12-h light, 12-h dark cycle. Water and food were provided ad libitum. At 28 days of age all chickens were bled by cardiac puncture and killed by cervical dislocation. Blood was collected into tubes containing EDTA, and plasma was prepared by centrifugation and frozen at −20°C until analyzed. Care and treatment of all chickens were approved by the Institutional Animal Care and Use Committee of the US Department of Agriculture.

2.2. IGF binding protein analysis

Plasma samples were diluted (1:10) with distilled H_2O and incubated for 10 min at 60°C in SDS–Tris–glycine loading buffer containing 10% glycerol and bromophenol blue, pH 7.0. Samples were subjected to discontinuous SDS-PAGE [6] through a 4% stacking gel and a 10% separating gel (Mini Protean II, 1.5-mm gels using a 15-lane format, BioRAD Laboratories, Hercules, CA). All buffers and gels were prepared without reducing agents. Molecular weight markers were prepared in loading buffer containing dithiothreitol. Experimental samples (equivalent to 3.0 μl of plasma) were loaded onto each gel along with three lanes of a common chicken plasma pool. The control chicken plasma pool was prepared from 8-week-old chickens and maintained at −80°C. On each gel, nine individual experimental samples were loaded and duplicate samples were run on separate gels. Following electrophoresis, proteins were transferred in Tris–glycine buffer (without methanol) onto 0.2-μm pore size pure nitrocellulose sheets with a Trans-Blot SD Semi-Dry Transfer System (BioRAD Laboratories). The nitrocellulose sheets were air-dried and IGF ligand blots were blocked with bovine albumin and NP-40, probed and developed essentially as described by Hossenlop et al. [4]. Each blot was incubated overnight with gentle rocking at 4°C with 250 000 cpm [^{125}I]cIGF-II, which was prepared as previously described [11]. Blots were exposed to X-ray film (XAR5, Kodak, Rochester, NY) and films were developed after 14 days. Distribution and characterization of IGF-II binding activity was similar to those previously reported [11]. IGF-II binding intensity (spot volume) was evaluated by laser densitometry (Computing Densitometer, Molecular Dynamics, Sunnyvale, CA). For each plasma sample (pooled control and experimental), the IGF binding activity (spot volume) was determined for each of the four distinct binding proteins at 70, 43, 30 and 24 kDa. Binding activities were averaged for the three control samples (pooled chicken plasma) which were run on each gel. The relative binding activity of each IGF-binding protein in the experimental samples was determined by dividing the individual spot volume by the average spot volume obtained from the corresponding IGF-binding protein of the pooled control plasma samples, which were on the same gel. Thus relative binding activity of experimental samples from different gels could be directly compared. Normalized data from duplicate samples were averaged and mean ± S.E.M. for the six sample are presented.

2.3. Hormone assays

Plasma IGF-I was determined with a homologous radioimmunoassay as previously described [10]. The concentration of chicken growth hormone was determined by homologous radioimmunoassay as previously described [24]. All hormone assays were conducted as single batches to remove inter-assay variation.

2.4. Statistical analysis

All data are reported as means ± S.E.M., $n = 6$ chickens per group. Data were analyzed by one-way

Table 1
Influence of dietary protein on body weight, feed intake and feed/gain ratio in 28-day-old broilers[a]

Dietary protein (%)	Final body weight (g)	Total feed intake (g)	Feed/gain ratio
12	645 ± 29^{b}	1152 ± 26^{b}	2.29 ± 0.12^{b}
21	975 ± 22^{c}	1386 ± 50^{c}	1.65 ± 0.07^{c}
30	956 ± 33^{c}	1393 ± 26^{c}	1.72 ± 0.10^{c}

[a] Values are means ± S.E.M. for each group ($n = 6$). Different superscripts in a column indicate that means are significantly different ($P < 0.05$).

ANOVA (GLM, Statmost, DataMost, Salt Lake City, UT) and means were compared by Tukey's Test. Differences between means are considered significant at $P < 0.05$.

3. Results

The body weights of chickens fed 21% crude protein were greater than chickens fed 12% protein (Table 1). No further increase in growth rate was observed when chickens were fed higher levels of dietary protein. Feeding low dietary protein was also associated with a reduction in feed intake. Feed/gain ratios were significantly lower for the chickens consuming 21 and 30% crude protein, compared with those consuming 12% protein.

Plasma levels of growth hormone were approximately 10-fold higher in chickens consuming 12% protein compared with those consuming 21 or 30% dietary protein (Table 2). In contrast, chickens consuming 12% dietary protein had concentrations of IGF-I which were approximately half that of chickens consuming 21 or 30% dietary protein.

An autoradiogram of an [^{125}I]cIGF-II ligand binding blot containing plasma samples from chickens fed different levels of dietary protein is shown in Fig. 1. Four regions of binding activity corresponding to 70, 43, 30 and 24 kDa were observed in all samples. Binding activity of duplicate samples associated with the four regions delineated by molecular weight was estimated by laser densitometry and is expressed as a fraction of the pooled control plasma samples which were run on each gel (Table 3). Birds consuming 12% dietary protein had less than 50% of the 43-kDa binding activity of birds consuming 21 or 30% dietary protein ($P < 0.01$); The 30-kDa binding activity was also lower ($P < 0.01$) in the 12% dietary protein group compared with those consuming higher levels of dietary protein. In contrast, 70- and 24-kDa binding activities were apparently not influenced by dietary protein intake levels.

4. Discussion

The influences of quality and quantity of dietary protein on growth of chickens are complex and involve not only regulation of amino acid availability for protein deposition, but also impacts upon lipid and energy substrate metabolism [16]. In most mammals, consumption of low protein diets is associated with elevated circulating growth hormone levels and a decrease in IGF-I concentrations [15,21]. Thus, under experimental conditions, there is a positive correlation between growth and circulating IGF-I concentrations. Likewise, decreased IGF-I levels have been previously reported with low protein intake in chickens [17]. Increased circulating growth hormone levels associated with low protein intake has also been reported [18]. While, the molecular control of secretion and dietary influences on circulating growth hormone levels are considered to be similar for chickens and mammals, neuroendocrine regulation of growth hormone synthesis and secretion differ markedly [3].

In chickens, the 43-kDa binding protein which appears as a doublet on SDS gels is apparently homologous to the mammalian IGF-BP3 protein [20,23]. This peptide represents the IGF binding portion of the high-molecular weight (150 kDa) IGF-binding protein complex [11]. In mammals, the majority of circulating IGFs are associated with this high molecular weight complex. Likewise, in chickens, it has been estimated that 70% of circulating IGF-I is associated with the high molecular weight IGF binding protein complex [1,2].

The present study represents the first successful attempt to identify and quantify all the major IGF-binding species in chicken plasma in response to low and high protein nutrition. These data indicate that a marginally low protein diet (12%) fed for 3 weeks was associated with a significant reduction in 43-kDa bind-

Table 2
Influence of dietary protein on growth hormone and IGF-I concentrations in serum of 28-day-old broilers[a]

Dietary protein (%)	Growth hormone (ng/ml)	IGF-I (ng/ml)
12	54.7 ± 11.0^{b}	12.3 ± 1.3^{b}
21	5.2 ± 0.6^{c}	24.8 ± 1.5^{c}
30	5.7 ± 1.1^{c}	30.7 ± 0.7^{c}

[a] Values are means ± S.E.M. for each group ($n = 6$). Different superscripts in a column indicate that means are significantly different ($P < 0.05$).

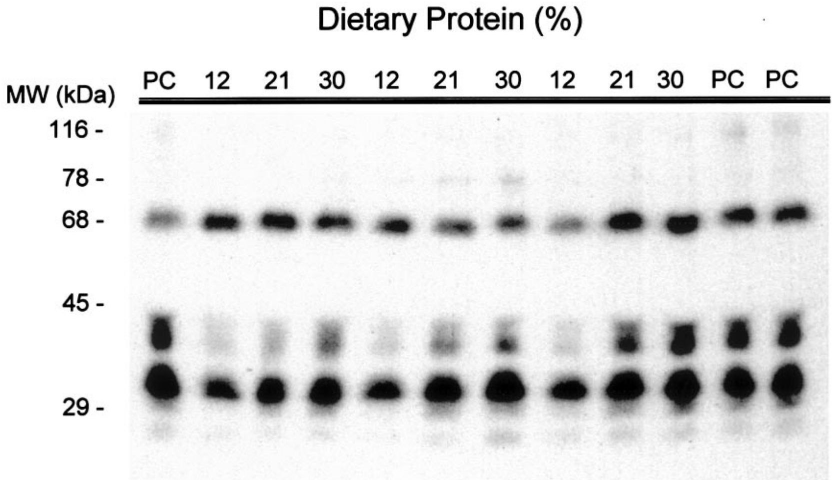

Fig. 1. Ligand blot of chicken plasma probed with [^{125}I]IGF-II. Samples (3 μl plasma) were subjected to electrophoresis and blotting as described in Section 2; PC, pooled control chicken plasma. The molecular mass of standards prepared in loading buffer containing 5 mM dithiothreitol were: B-galactosidase (116 kDa), transferrin (78 kDa), bovine serum albumin (68 kDa), ovalbumin (45 kDa) and carbonic anhydrase (29 kDa).

ing activity. Moreover, circulating levels of IGF-I appear to be linearly correlated with the observed levels of the 43-kDa IGF binding protein. Decreased circulating IGF-BP3 and IGF-I was also reported in rats fed low protein (5%) or protein-free diets [7,22]. In addition, in similar-aged egg laying chickens, a tendency towards lower 40-kDa IGF-I binding activity was also reported [9]. Thus, with regard to the major IGF binding species under apparent regulation by IGF, mammals and chickens appear to respond similarly to dietary protein stress.

Present data indicate that in addition to the marked decrease in circulating IGF-BP3 (43-kDa binding activity), 30-kDa IGF-binding protein activity is also decreased in broiler chickens following low protein consumption. The 30-kDa binding activity band probably represents both IGFBP-1 and IGFBP-2 [2,14], since these proteins would not be completely resolved by ligand blot analysis following SDS Page. In contrast, Leili and Scanes [9] reported a transient, but not long-term increase in 30-kDa IGF binding activity in Leghorn chickens. Additionally, Kita and coworkers [5] also reported a slight increase in 30-kDa IGF binding activity following 1 week consumption of a low protein diet in broilers. The differences reported may be methodological in nature, as in our hands, 30-kDa IGF binding activity is a prominent band observed in ligand blots and represents a major proportion of the IGF binding activity, whether blots are probed with radiolabeled IGF-I or IGF-II [11]. In contrast, data presented by these investigators indicated that in normally-fed control birds, 30-kDa IGF-binding activity was barely detectable and was increased in protein deficient and feed-restricted birds [5,8,9]. In agreement with data presented here, a decrease in circulating 30-kDa IGF-binding activity was reported in protein restricted rats [7]. These same investigators determined that IGF-BP1 mRNA and IGF-BP2 mRNA were increased in the liver, while IGFBP-3 mRNA was decreased [7]. Taken together, these data indicate that the integrated nutritional regulation of circulating IGF binding protein levels is complex involving, on one hand, the possibility of enhanced or diminished peptide synthetic capacity, which is coupled with dynamic protein turnover processes that differ for individual binding proteins [7].

In the present study, binding activity of the 70-kDa IGF-II-specific binding protein was apparently not influenced by dietary protein intake. While specific information is lacking concerning the extent of saturation of the 70-kDa binding protein with IGF-II, it is clear that IGF-II binds to the 70-kDa binding protein in vivo [11]. Although the plasma concentration of IGF-II was not determined, the present data may suggest that there

Table 3
Influence of dietary protein on [^{125}I]IGF-II binding activity in plasma of 28-day-old broilers[a]

Dietary protein (%)	Relative absorbance units			
	70 kDa	43 kDa	30 kDa	24 kDa
12	1.27 ± 0.10	0.317 ± 0.054^b	0.687 ± 0.082^b	0.912 ± 0.267
21	1.60 ± 0.20	0.696 ± 0.124^c	1.19 ± 0.11^c	0.948 ± 0.252
30	1.48 ± 0.11	0.805 ± 0.091^c	1.14 ± 0.07^c	1.02 ± 0.17

[a] Values are means ± S.E.M. for each group ($n = 6$). Samples were run in duplicate and each value represents the spot volume of the individual experimental sample/average spot volume of the reference control chicken plasma sample. Different superscripts in a column indicate that means are significantly different ($P < 0.05$).

is limited influence of dietary protein on IGF-II levels, due to the lack of change in the specific 70-kDa IGF-II binding protein. Moreover, we have recently determined that a low protein diet did not alter IGF-II concentrations in broiler chickens [13]; similar results were reported for broilers following feeding a low protein diet for 1 week [5]. The overall role of IGF-II in growth processes has not been well defined in chickens, and studies where circulating IGF-II concentrations have been quantified are limited [5,8,13,19]. In addition, a distinct receptor for IGF-II in chickens has not been identified [26]. Clearly, the availability of a homologous assay for chicken IGF-II will help in elucidating the regulation of IGF-II and its interaction with its 70-kDa binding protein [13]. Studies are currently being conducted to determine how alterations in IGF-binding proteins associated with low dietary protein intake influences circulating IGF-II and turnover of IGF-I and IGF-II in vivo.

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